Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

Claims 1-83 (Canceled).

84. (Previously presented) A method of selecting polynucleotides which encode an antigen-specific human immunoglobulin molecule, comprising:

(a) introducing into a population of mammalian host cells capable of expressing said immunoglobulin molecule and permissive for vaccinia virus infectivity, a first library of polynucleotides encoding, through operable association with a transcriptional control region, a plurality of first immunoglobulin subunit polypeptides, each first immunoglobulin subunit polypeptide comprising:

(i) a first immunoglobulin constant region selected from the group consisting of a heavy chain constant region and a light chain constant region,

(ii) an immunoglobulin variable region selected from the group consisting of a heavy chain variable region and a light chain variable region, wherein said variable region corresponds to said first constant region, and

(iii) a signal peptide capable of directing cell surface expression or secretion of said first immunoglobulin subunit polypeptide,

wherein said first library is constructed in a vaccinia virus vector, provided said first library is not constructed by traditional homologous recombination;

(b) introducing into said host cells a second library of polynucleotides encoding, through operable association with a transcriptional control region, a plurality of second immunoglobulin subunit polypeptides, each comprising:

(i) a second immunoglobulin constant region selected from the group consisting of a heavy chain constant region and a light chain constant region, wherein said second immunoglobulin constant region is not the same as said first immunoglobulin constant region,

(ii) an immunoglobulin variable region selected from the group consisting of a heavy chain variable region and a light chain variable region, wherein said variable region corresponds to said second constant region, and

(iii) a signal peptide capable of directing cell surface expression or secretion of said second immunoglobulin subunit polypeptide,

wherein said second immunoglobulin subunit polypeptide is capable of combining with said first immunoglobulin subunit polypeptide to form an immunoglobulin molecule, and wherein said second library is constructed in a vaccinia virus vector, provided said second library is not constructed by traditional homologous recombination;

(c) permitting expression of immunoglobulin molecules, from said host cells;

(d) contacting said immunoglobulin molecules with an antigen and detecting specific antigen-antibody complexes; and

(e) recovering vaccinia virus vectors containing polynucleotides of said first library which encode immunoglobulin subunit polypeptides which, as part of an immunoglobulin molecule, are specific for said antigen.

85. (Withdrawn) The method of claim 84, wherein the vaccinia virus vectors containing said second library of polynucleotides are rendered incapable of producing infectious vaccinia virus virions in said host cells.

86. (Withdrawn and previously presented) The method of claim 85, further comprising:

(f) introducing the vaccinia virus vectors recovered in (e) into a population of mammalian host cells capable of expressing said immunoglobulin molecule and permissive for vaccinia virus infectivity;

(g) introducing into said host cells said second library of polynucleotides;

(h) permitting expression of immunoglobulin molecules, from said host cells;

(i) contacting said immunoglobulin molecules with said antigen and detecting specific antigen-antibody complexes; and

(j) recovering vaccinia virus vectors containing polynucleotides of said first library which encode immunoglobulin subunit polypeptides which, as part of an immunoglobulin molecule, are specific for said antigen.

87. (Withdrawn and previously presented) The method of claim 86, further comprising repeating steps (f)-(j) one or more times, thereby enriching for polynucleotides of said first library which encode a first immunoglobulin subunit

polypeptide which, as part of an immunoglobulin molecule, specifically binds said antigen.

88. (Previously presented) The method of claim 84, further comprising isolating the immunoglobulin subunit polypeptide-encoding polynucleotides contained in the vaccinia virus vectors recovered from said first library.

89. (Previously presented) The method of claim 88, further comprising:

(k) introducing into a population of mammalian host cells capable of expressing said immunoglobulin molecule and permissive for vaccinia virus infectivity vaccinia virus vectors containing said second library of polynucleotides, wherein said vaccinia virus vectors are infectious;

(l) introducing into said host cells vaccinia virus vectors containing those polynucleotides isolated from said first library, wherein the vaccinia virus vectors containing said isolated polynucleotides are rendered incapable of producing infectious vaccinia virus vectors in said host cells;

(m) permitting expression of immunoglobulin molecules from said host cells;

(n) contacting said immunoglobulin with said specific antigen and detecting specific antigen-antibody complexes; and

(o) recovering vaccinia virus vectors containing polynucleotides of said second library which encode immunoglobulin subunit polypeptides which, as part of an immunoglobulin molecule, are specific for said antigen.

90. (Currently Amended) The method of claim 89, further comprising:

(p) introducing the vaccinia virus vectors recovered in (o) into a population of mammalian host cells capable of expressing said immunoglobulin molecule and permissive for vaccinia virus infectivity;

(q) introducing into said host cells vaccinia virus vectors containing those polynucleotides isolated from said first library, wherein the vaccinia virus vectors containing said isolated polynucleotides are rendered incapable of producing infectious vaccinia virus ~~visions~~ virions in said host cells;

(r) permitting expression of immunoglobulin molecules from said host cells;

(s) contacting said immunoglobulin molecules with said antigen and detecting specific antigen-antibody complexes; and

(t) recovering vaccinia virus vectors containing polynucleotides of said second library which encode immunoglobulin subunit polypeptides which, as part of an immunoglobulin molecule, are specific for said antigen.

91. (Previously presented) The method of claim 90, further comprising repeating steps (p)-(t) one or more times, thereby enriching for polynucleotides of said second library which encode a second immunoglobulin subunit polypeptide which, as part of an immunoglobulin molecule, specifically binds said antigen.

92. (Previously presented) The method of claim 91, further comprising isolating the immunoglobulin subunit polypeptide-encoding polynucleotides contained in the vaccinia virus vectors recovered from said second library.

93. (Previously presented) A method of producing a first polynucleotide and a second polynucleotide which encode an antigen-specific human immunoglobulin

molecule comprising combining a first polynucleotide and a second polynucleotide isolated according to claim 92.

94. (Previously presented) A method of producing a host cell which expresses an antigen-specific human immunoglobulin molecule comprising introducing the first and second polynucleotides produced as recited in claim 93 into a mammalian host cell capable of expressing said first and second polynucleotides.

95. (Previously presented) A method of producing an antigen-specific human immunoglobulin molecule comprising:

culturing a host cell produced according to the method of claim 94 under conditions wherein said first and second polynucleotides are expressed; and

recovering said antigen-specific human immunoglobulin molecule.

96. (Previously presented) The method of claim 84, wherein said plurality of first immunoglobulin subunit polypeptides are immunoglobulin heavy chains.

97. (Previously presented) The method of claim 84, wherein said plurality of first immunoglobulin subunit polypeptides are immunoglobulin light chains.

98. (Withdrawn) The method of claim 85, wherein said host cells are infected with said first library at an MOI ranging from about 1 to about 10, and wherein said second library is introduced under conditions which allow up to 20 vaccinia virus vectors of said second library to be taken up by each infected host cell.

99. (Previously presented) The method of claim 89, wherein said host cells are infected with said second library at an MOI ranging from about 1 to about 10.

100. (Withdrawn) The method of claim 84, wherein said transcriptional control region comprises a poxvirus promoter.

101. (Withdrawn) The method of claim 100, wherein said promoter is a vaccinia virus p7.5 promoter.

102. (Withdrawn) The method of claim 100, wherein said promoter is a vaccinia MH-5 promoter.

103. (Previously presented) The method of claim 84, wherein said transcriptional control region comprises a T7 phage promoter active in cells in which T7 RNA polymerase is expressed.

104. (Withdrawn) The method of claim 84, wherein said transcriptional control region comprises a transcriptional termination region.

105. (Withdrawn) The method of claim 84, wherein said vaccinia virus vector is attenuated.

106. (Withdrawn) The method of claim 105, wherein said vaccinia virus vector is deficient in D4R synthesis.

107. (Previously presented) The method of claim 84, wherein said first library of polynucleotides is constructed by a method comprising:

(a) cleaving an isolated vaccinia virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising said polynucleotides which encode said plurality of immunoglobulin heavy or light chains, or fragments thereof through operable association with a transcription control region,

flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to a terminal portion of said first viral fragment and said 3' flanking region is homologous to a terminal portion of said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable vaccinia virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a mammalian host cell permissive for vaccinia virus infectivity under conditions wherein said transfer plasmids and said viral fragments undergo *in vivo* homologous recombination, thereby producing a viable modified vaccinia virus genome comprising a polynucleotide which encodes an immunoglobulin heavy chain or an immunoglobulin light chain; and

(d) recovering said modified vaccinia virus genome.

108. (Previously presented) The method of claim 107, wherein said vaccinia virus genome is selected from the group consisting of a v7.5/tk virus genome and a vEL/tk virus genome

109. (Previously Presented) The method of claim 107, wherein said first viral fragment and said second viral fragment are generated by cleaving one or more unique restriction sites selected from the group consisting of a unique NotI restriction site, a unique ApaI restriction site, and a combination of a unique NotI restriction site and a unique ApaI restriction site, in the tk gene of said vaccinia virus genome.

110. (Previously presented) The method of claim 84, wherein said second library of polynucleotides is constructed by a method comprising:

(a) cleaving an isolated vaccinia virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising said polynucleotides which encode said plurality of immunoglobulin light or heavy chains, or fragments thereof through operable association with a transcription control region, flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to a terminal portion of said first viral fragment and said 3' flanking region is homologous to a terminal portion of said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable vaccinia virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a mammalian host cell permissive for vaccinia virus infectivity under conditions wherein said transfer plasmids and said viral fragments undergo homologous recombination, thereby producing a viable modified vaccinia virus genome comprising a polynucleotide which encodes an immunoglobulin light chain or an immunoglobulin heavy chain; and

(d) recovering said modified vaccinia virus genome.

111. (Previously presented) The method of claim 110, wherein said vaccinia virus genome is selected from the group consisting of a v7.5/tk virus genome and a vEL/tk virus genome

112. (Previously presented) The method of claim 110, wherein said first viral fragment and said second viral fragment are generated by cleaving one or more unique

restriction sites selected from the group consisting of a unique NotI restriction site, a unique ApaI restriction site, and a combination of a unique NotI restriction site and a unique ApaI restriction site, in the tk gene of said vaccinia virus genome.

113. (Previously presented) The method of claim 96, wherein said immunoglobulin heavy chains are a secreted form of an immunoglobulin heavy chain.

114. (Previously presented) The method of claim 113,

wherein vaccinia virus vectors containing said first library of polynucleotides are divided into a plurality of virus pools, and each virus pool is infected into a separate population of mammalian host cells to form a plurality of host cell pools;

wherein said host cell pools are cultured such that immunoglobulin molecules are expressed and secreted into the culture medium containing said host cell pools to form a plurality of immunoglobulin pools;

wherein said immunoglobulin pools are contacted with said antigen, and specific antigen-antibody complexes are detected; and

wherein vaccinia virus vectors are recovered from those host cell pools which expressed immunoglobulin pools from which specific antigen-antibody complexes were detected.

115. (Previously presented) The method of claim 114, further comprising:

(a) dividing said recovered vaccinia virus vectors into a plurality of virus sub-pools and infecting each virus sub-pool into a separate population of mammalian host cells to form a plurality of host cell sub-pools;

(b) culturing said host cell sub-pools such that immunoglobulin molecules are expressed and secreted into the culture medium containing said host cell sub-pools to form a plurality of immunoglobulin sub-pools;

(c) contacting said immunoglobulin sub-pools with said antigen, and detecting specific antigen-antibody complexes; and

(d) recovering vaccinia virus vectors from those host cell sub-pools which expressed immunoglobulin sub-pools from which specific antigen antibody complexes were detected.

116. (Previously presented) The method of claim 115, further comprising repeating steps (a)-(d) one or more times, thereby enriching for polynucleotides of said first library which encode a first immunoglobulin subunit polypeptide which, as part of an immunoglobulin molecule specifically binds said antigen.

117. (Previously presented) The method of claim 97, wherein said second immunoglobulin subunit polypeptides are a secreted form of an immunoglobulin heavy chain.

118. (Previously presented) The method of claim 117,

wherein vaccinia virus vectors containing said first library of polynucleotides are divided into a plurality of virus pools, and each virus pool is infected into a separate population of mammalian host cells to form a plurality of host cell pools;

wherein said host cell pools are cultured such that immunoglobulin molecules are expressed and secreted into the culture medium containing said host cell pools to form a plurality of immunoglobulin pools;

wherein said immunoglobulin pools are contacted with said antigen, and specific antigen-antibody complexes are detected; and

wherein vaccinia virus vectors are recovered from those host cell pools which expressed immunoglobulin pools from which specific antigen antibody complexes were detected.

119. (Previously presented) The method of claim 118, further comprising:

(a) dividing said recovered vaccinia virus vectors into a plurality of virus sub-pools and infecting each virus sub-pool into a separate population of mammalian host cells to form a plurality of host cell sub-pools;

(b) culturing said host cell sub-pools such that immunoglobulin molecules are expressed and secreted into the culture medium containing said host cell sub-pools to form a plurality of immunoglobulin sub-pools;

(c) contacting said immunoglobulin sub-pools with said antigen, and detecting specific antigen-antibody complexes; and

(d) recovering vaccinia virus vectors from those host cell sub-pools which expressed immunoglobulin sub-pools from which specific antigen antibody complexes were detected.

120. (Previously presented) The method of claim 119, further comprising repeating steps (a)-(d) one or more times, thereby enriching for polynucleotides of said first library which encode a first immunoglobulin subunit polypeptide which, as part of an immunoglobulin molecule specifically binds said antigen.

121. (Previously presented) The method of claim 114, wherein said detecting is by ELISA.

122. (Previously presented) The method of claim 118, wherein said detecting is by ELISA.

Claims 123-126 (Canceled).

127. (Previously presented) A method of producing a library of polynucleotides which encode a plurality of human immunoglobulin subunit polypeptides in a vaccinia virus vector comprising:

(a) cleaving an isolated vaccinia virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising a plurality of polynucleotides encoding, through operable association with a transcription control region, a plurality of immunoglobulin subunit polypeptides, flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to said first viral fragment and said 3' flanking region is homologous to said second viral

fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a mammalian host cell under conditions wherein said transfer plasmids and said viral fragments undergo *in vivo* homologous recombination, thereby producing a plurality of viable modified virus genomes, each comprising a polynucleotide which encodes an immunoglobulin subunit polypeptide; and

(d) recovering said plurality of modified virus genomes.

128. (Previously presented) The method of claim 127 wherein each human immunoglobulin subunit polypeptide comprises:

(a) a first immunoglobulin constant region selected from the group consisting of a heavy chain constant region and a light chain constant region;

(b) an immunoglobulin variable region corresponding to said first constant region; and

(c) a signal peptide capable of directing cell surface expression or secretion of said first immunoglobulin subunit polypeptide.

129. (Previously presented) The method of claim 84, wherein step (e) further comprises recovering vaccinia virus vectors containing polynucleotides of said second library which encode immunoglobulin subunit polypeptides which, as part of an immunoglobulin molecule are specific for said antigen.

130. (Previously presented) The method of claim 129, further comprising isolating the immunoglobulin subunit polypeptide-encoding polynucleotides contained in the vaccinia virus vectors recovered from said second library.

131. (Previously presented) A method of producing a first polynucleotide and a second polynucleotide which encode an antigen-specific human immunoglobulin molecule comprising combining a first polynucleotide and a second polynucleotide isolated according to claim 130.